NOTES ON THE USE OF Ca^{45} IN DETERMINING LEAF THICKNESS

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Abstract

The usefulness of leaf thickness as taxonomic character can be enhanced by a simple, rapid method of determining dry leaf lamina thickness. Several experiments were conducted with $Calcium^{45}$ testing the principle that thicker laminae absorb more soft beta particles than thinner laminae. By measuring the changes in particle intensity, mass is obtained; to the degree that thickness correlates with mass, one has determined the thickness. Shards of *Magnolia grandiflora* L., *Asimina triloba* (L.) Dunal, and *Vicia americana* Willd. were used. Results of these tests indicate that there is a correlation between the amount of beta particles passing through the laminae of dry leaves and the thickness of the laminae.

Introduction

Leaf characters are commonly used in taxonomic treatments. Leaf thickness, if reported, usually is determined by inspection. Aside from this empirical method, there is apparently no simple method of ascertaining thickness. Thickness has been determined by 1) use of cursory examination, 2) use of either freehand or microtome sections in conjunction with an optical-measurement system, 3) use of a micrometer or, 4) use of a punch-weigh system. The section-optical-measurement system is not simple; the micrometer method results in large error; and the punch-weighing system is time consuming and cannot be applied to leaves with narrow laminae. The usefulness of leaf thickness as a taxonomic character would be augmented if objective measurement techniques were available.

Radioactive isotopes, as the sources of beta particles for determining thickness, have had practical application in industry. Zumwalt (1954) reported two common uses of beta particles in industry. These are the determination of the thickness of continuously moving materials, and the concentrations of solutions. Measurements are based on two principles. The absorption of the beta particles as they pass through a material

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provides an indication of the thickness of the material. The degree of backscattering of beta particles from a substance also can be used to determine thickness.

The principle of absorption of beta particles as they pass through a material is applicable to leaf studies. The few investigations that have been published are concerned with water content of leaves, leaf thickness being a complicating variable (Nakayama and Ehrler, 1964; and Yamada, et al., 1958). No reference has been found to studies undertaken from the taxonomic point of view.

When a leaf is irradiated with beta particles, the intensity of the rays decreases as a result of interaction with the leaf. Thickness may be calculated from the equation

$$I=I_o e^{-\mu d}$$

where d is the weight per unit area and μ the absorption coefficient (a constant, which is determined only by the maximum energy of the beta particles and is peculiar to the nuclide used); for Ca^{45} it is 0.128. The logarithmic constant e is 2.718, I_o is the unmodified intensity, and I is the modified intensity. When d=0, $I=I_o$. Therefore when thickness is measured by using beta particles, the expression is not in units of distance, but in units of weight per unit area, e.g., milligrams per square centimeter. The results can also be reported as counts per unit of time from a standardized source. By inserting the leaf between the radiation source and the Geiger-Müller (GM) tube, the intensity of the radiation changes proportionately to the leaf mass. Thus by measuring such intensity changes, mass is obtained; to the degree that thickness correlates with mass, one has determined the thickness.

Not all beta sources are amenable to herbarium leaf studies. Sources such as $Strontium^{90}$, $Yttrium^{90}$, and Radium D&E (all used in industry) are not useful in leaf studies because of the strong penetration capacity of their beta particles. Sources such as $Calcium^{45}$ and $Sulpher^{35}$ are applicable because of the weaker penetration capacity of their beta rays. The former have been labelled hard sources, the latter soft sources.

Materials and Methods

A Radiation Counter Laboratories Scaler-ratemeter, model 20324 was used to measure and record the beta radiation (Fig. 1). The lead shields covering the plastic planchets (Fig. 2) holding the sources contained 300 milligrams of lead per square centimeter. This thickness of lead absorbed the radiation from all sources tested. The holes drilled through the shields were 1/16 inch in diameter. These holes allowed the passage of beta rays from the source through the shield and test material to the GM tube. Since the isotopes were not uniformly distributed in the matrices, the shields were taped to the planchets. The distance between the source and the test material was 2.5 mm.; the distance between the tested material and the GM tube, 11 mm. The background count averaged 9.2 counts per minute with a range of 11.8 to 6.3 counts per minute. The high voltage varied from 810 to 830, usually holding steady at 820, a setting recommended by the manufacturer.

Sixty herbarium sheets of *Vicia americana* and its varieties (Gunn, 1968) were selected to represent the variation of leaflet thickness in its North American range. The leaflets were selected at random from these sheets. The count per minute from the open source was ± 2700.

A single leaflet of *Vicia americana* Willd. was tested for one half hour; readings were taken every minute using a 1-hole plate. When the resulting information was analyzed by means of maximum curvature, it was found that a 3-minute count interval was sufficient.

The thickness of *V. americana* leaflet shards was also measured by using a compound microscope equipped with an ocular micrometer and an oblique above-stage microscope light. The measurements were recorded in increments of 11.1 microns, rounded to the nearest whole number.

Results and Discussion

Saran Wrap with Ca^{45} as the beta producing isotope was used to test the equation $I=I_o e^{-\mu d}$. In a sequence of tests, layers of Saran Wrap were added (from 1 to 13) to the top of a one-hole plate, and readings were taken every 3 minutes. In Fig. 3 the layers of Saran Wrap were plotted against the log of the counts per minute producing nearly a straight line. These results illustrate that thickness can be determined by counting the beta particles that are not absorbed by the test material. The linear arrangement of the averaged counts per minute in Fig. 3 proves this point. The extension of this concept from a homogeneous material (Saran Wrap) to a hetergeneous material (leaf laminae) was tested.

Radium D&E, Carbon 14, and Calcium 45 were surveyed with shards of two test leaves taken from herbarium (dry) material possessing obvious differences in thickness, *Magnolia grandiflora* (magnolia) and *Asimina triloba* (pawpaw). Of the three isotopes used, only Ca^{45} gave results which were commensurate with the 8.1 thickness ration of dried magnolia and pawpaw. The results from the Radium D&E test were inconclusive, since there was more intra- than inter-leaf variation. Readings obtained from C^{14} were too close to the background count to be usable.

Before testing Ca^{45} on the other leaves, lead shields with 1, 2, and 3 holes (Fig. 2) were used with the magnolia and paw-paw leaves. In Fig. 4 each dot represents five, 3-minute counts averaged. Based on the results of the 3-hole test when compared to the 1-hole test, the Ca^{45} concentration was trebled in *Vicia americana* leaflet tests. This increased the 1-minute count through a 1/16 inch diameter aperature from ± 385 to ± 2700 counts per minute, a seven-fold increase.

Magnolia shards with the red indumentum of hairs intact absorbed as much beta radiation as the same shards when denuded. This indicates that pubescence is not a factor affecting the outcome of this type of thickness determination.

When the leaflets of *Vicia americana* were introduced into the system, the counts ranged from 846 (the thickest leaflet) to 1874 (the thinnest leaflet). These counts were converted to logs and plotted against the measurements recorded in microns obtained from the optical system. These results are given in Fig. 5. The larger dots represent the 95 percent confidence limits of the population means. The means are represented by the smaller dots. The decrease in the counts per minute with the increase of leaflet thickness indicates a direct relationship between leaflet thickness and the amount of absorbed beta particles. A comparison of Figs. 3 and 5 reveals that while the leaflet means are more variable than the Saran Wrap means, the test did measure leaflet thickness. An analysis of the leaflet data indicates that 57 percent (r^2=56.94) of the variation in the counts per minute can be attributed to the thickness of the leaflets.

The measurements in microns are at best an estimate. Therefore, the 57 percent correlation figure may be low because of errors in the measurement system. Additional tests on other leaves using other standards would help to establish the correct correlation between true leaf thickness and the amount of absorbed beta particles.

Literature

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Zumwalt, L. R. 1954. The best performance from beta gauges. Nucleonics 12(1):55-58.

Mention of material by trade-name implies no preference over similar equipment made by other manufacturers.

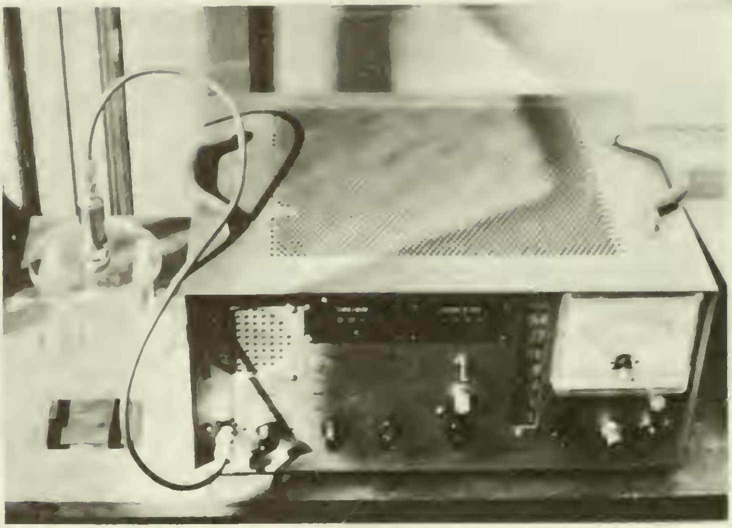

Fig. 1. Radiation Counter Laboratories Scaler-ratemeter, model 20324.

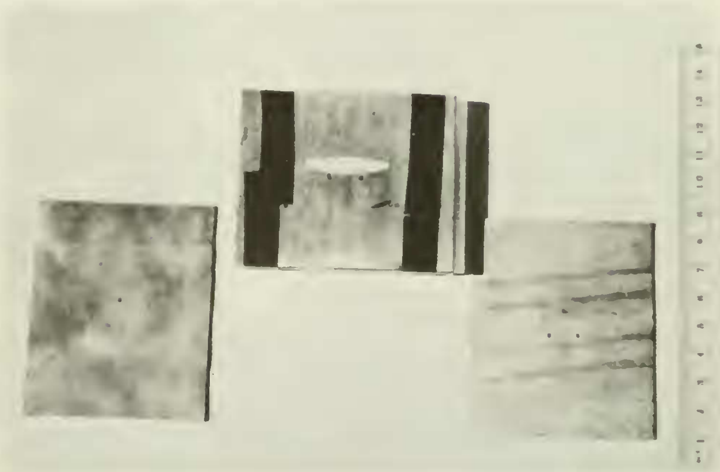

Fig. 2. A 2-hole lead shield taped to a planchet and carrying slide with a *Vicia americana* leaflet.

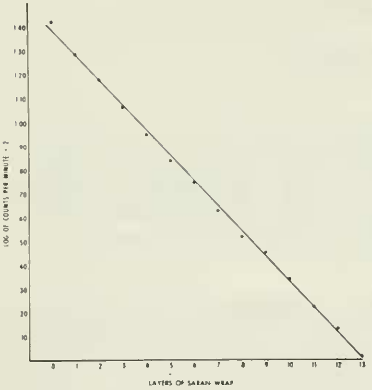

Fig. 3. Layers of Saran Wrap plotted against logs of counts per minute plus 2. Ca^{45} count per minute was ± 2700.

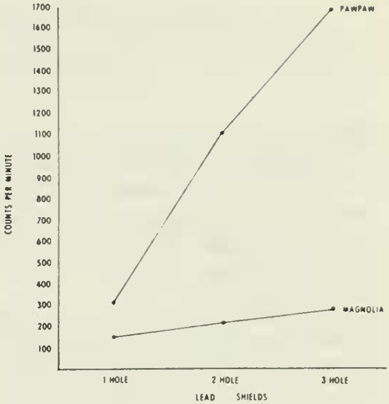

Fig. 4. Pawpaw and magnolia leaf shards tested with 1-, 2-, and 3-hole lead shields. The Ca^{45} count per minute was ± 305.

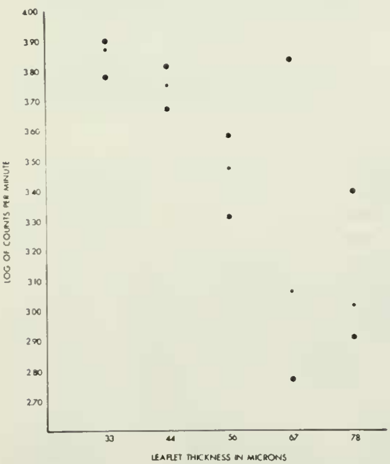

Fig. 5. *Vicia americana* leaflets thickness in microns plotted against the logs of the count per minute. The Ca^{45} count per minute was ± 2700. The larger dots represent the 95% confidence limits of the population means. The smaller dots represent the population means.